claim 40.

The Amendments

Claims 41, 46, 47, 48, and 49 are amended to specify that the recited mutation "attenuates the bacterium." Support for this amendment is found, *e.g.*, at page 8, line 24, to page 9, line 1 of the specification:

> Also provided by the present invention are mutant strains made by the disclosed method of site-directed mutagenesis. One such mutant (NADC-D60 aroA$^-$) has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA, on December 2, 1993, under the terms of the Budapest Treaty as Accession No. ATCC 55518. Such mutants can provide the veterinary arts with attenuated, live strains of *P. haemolytica* which are suitable for vaccines to induce protective immunity against *P. haemolytica* infection. For vaccine production, it is desirable that the mutation which attenuates the *P. haemolytica* be an essentially non-reverting mutation.

The following amendments are made because claim 34 is canceled. Claims 46-49 are amended to be in independent form. Claim 40, which recites "*P. haemolytica* ATCC 55518," is amended to be dependent on claim 41; claim 41 recites an *aro*A mutation, and ATCC 55518 is a bacterium which comprises an *aro*A mutation.

Claims 43 and 44 are amended to recite that the claimed vaccines are "formulated for" particular routes of administration. New claims 52, 53, 55, 56, 58, 59, 61, and 62 also contain this recitation. Support for the recitation is found in the specification at page 9, lines 18-22: "[i]t is desirable that the *P. haemolytica* vaccines of the invention be administered by the intranasal or intratracheal route, but subcutaneous, intramuscular, intravenous injection also may be used. Suitable formulations and techniques are taught by Kucera U.S. 4,335,106, Gilmour U.S. 4,346,074, and Berget U.S. 4,957,739." New claims 51-62 mirror canceled claims 35, 38, and

39 for each of the now independent claims 46-49.

The Rejection of Claims 38, 39, 43, and 44 Under 35 U.S.C. § 112, second paragraph

Claims 38, 39, 43, and 44 stand rejected under 35 U.S.C. § 112, second paragraph. The Office Action mailed November 11, 2000 asserts that the limitations of claims 38, 39, 43, and 44 do not further limit the claims from which these claims depend. Claims 38 and 39 are canceled, rendering their rejection moot. Applicants respectfully traverse the rejection of claims 43 and 44.

Claims 43 and 44 have been amended to specify that the claimed vaccines are "formulated for" particular forms of administration. Claims 43 and 44 as amended are properly dependent. Applicants therefore respectfully request withdrawal of this rejection.

The Rejection of Claims 34, 38, 39, 46, and 47 Under 35 U.S.C. § 102(b)

Claims 34, 38, 39, 46, and 47 stand rejected under 35 U.S.C. § 102(b) as anticipated by Cruz *et al.* Cruz *et al.* is cited as teaching internal deletions in *lkt*A and *lkt*C of *P. haemolytica.* Claims 34, 38, and 39 are canceled, rendering their rejection moot. Applicants respectfully traverse the rejection of claims 46 and 47.

Amended claim 46 and newly added claims 51-53 recite a vaccine comprising a *P. haemolytica* bacterium comprising a mutation in a leukotoxin C gene. Amended claim 47 and newly added claims 54-56 recite a vaccine comprising a *P. haemolytica* bacterium comprising a mutation in a leukotoxin A gene. The Office Action asserts that Cruz *et al.* discloses "strains of Pasteurella haemolytica which were modified through a series of internal deletions in the lktA (leukotoxin-A) gene and leukotoxin C gene." Office Action at page 3, item 4. The Office

Action therefore concludes that Cruz *et al.* discloses strains of *P. haemolytica* that inherently anticipate claims 46 and 47.

Cruz *et al.* does not anticipate claims 46-47 and 51-53 because Cruz *et al.* teaches an *E. coli* host comprising mutated *P. haemolytica* DNA, whereas the claims require *P. haemolytica* bacteria which comprise a mutation in a leukotoxin A or C gene. Applicants do not dispute that Cruz *et al.* discloses deletions of the *P. haemolytica* leukotoxin A and C genes. Claims 46 and 51-53, however, require a *P. haemolytica* bacterium comprising a mutation in the leukotoxin C gene. Similarly, claims 47 and 54-56 require a *P. haemolytica* bacterium comprising a mutation in the leukotoxin A gene. Cruz *et al.* does not disclose strains of *P. haemolytica* comprising a mutation in a leukotoxin A gene. See pages 7-8 of the Appeal Brief filed August 7, 2000. As explained below, Cruz *et al.* also does not disclose strains of *P. haemolytica* comprising a mutation in a leukotoxin C gene. Because Cruz *et al.* does not disclose such bacteria, it cannot anticipate claims 46, 51-54, 47, or 54-56.

Cruz *et al.* discloses deletions within the *lktCA* locus (Fig. 1). These deleted genes, however, are present in "pYFC19." See the legend to Fig. 1: "Deletions within the *lktCA* locus of pYFC19." "pYFC19" is not a strain of *P. haemolytica*. It is a plasmid. The "Experimental Procedures" section of Cruz *et al.* states that pYFC19 is a plasmid at page 1938, column 1, first full paragraph:

> Plasmids which contains internal deletions of the *lktA* gene were derived from pYFC19 (Chang *et al.*, 1987) in which the *lktCA* genes are expressed under *lacPO* control.

Cruz *et al.* teaches that the pYFC19 plasmids which contain deletions of the *lkt*A gene are expressed in an *E. coli* host:

> Eight derivatives of pYFC19 were generated which contains

> internal deletions of the *lktA* gene (Fig. 1). Each mutant plasmid was found to express a truncated form of the LktA protein of the expected molecular weight **when present in the *E. coli* host, TB1** (Fig. 2).

Cruz *et al.*, page 1934, first column, second full paragraph, emphasis added. See also the legend to Fig. 2:

> Western blot analysis of the mutant LktA proteins **expressed from the deletion plasmids**. The leukotoxin secreted by *P. haemolytica* is shown in lane 1. The remaining samples were trichloroacetic acid precipitates of ***E. coli* harbouring** pHG165 (lane 2), pYFC19 (lane 3), plktCAd8 (lane 4), plktCAd2 (lane 5), plktCAd3 (lane 6), plktCAd4 (lane 7), plktCAd5 (lane 8), plktCAd7 (lane 9), plktCAd6 (lane 10), plktCAd1 (lane 11), and plktCd1Ad1 (lane 12).

Cruz *et al.*, page 1934, emphasis added. An *E. coli* host comprising a plasmid which contains a mutated *lkt*CA locus is not "a *P. haemolytica* bacterium which comprises a mutation in a leukotoxin C gene," as required by claims 46 and 51-54. Nor is the disclosed *E. coli* host "a *P. haemolytica* bacterium which comprises a mutation in a leukotoxin A gene," as required by claims 47 and 54-56.

Applicants also do not dispute that Cruz *et al.* discloses a strain of *P. haemolytica*, biotype A, serotype 1. Paragraph bridging pages 1937 and 1938. The disclosed strain, however, expresses native (*i.e.*, wild-type) leukotoxin protein. The section entitled "Preparation of native and truncated toxin proteins" on page 1938 describes the preparation of native leukotoxin from *P. haemolytica* (see the last paragraph of column 1). This section also describes the preparation of truncated, leukotoxin proteins expressed by the pYFC19 plasmids: "Deleted forms of the LktA protein were prepared by growing TB1 [the *E. coli* host] harbouring the various plasmids to an OD_{550} of >2.0." Page 1938, column 2, first paragraph. A *P. haemolytica* bacterium which expresses native leukotoxin does not meet the limitations of any of claims 46, 51-53, 47, or 54-

57, because each of these claims requires a *P. haemolytica* bacterium comprising a mutation in a leukotoxin gene.

There is simply no teaching in Cruz *et al.* of a *P. haemolytica* bacterium comprising a mutation in a leukotoxin A or C gene. Thus, Cruz *et al.* cannot anticipate claims 46, 51-53, 47, or 54-57. Applicants respectfully request withdrawal of this rejection.

The Rejection of Claims 34, 35, and 38-50 Under 35 U.S.C. § 112, second paragraph

Claims 34, 35, and 38-50 stand rejected under 35 U.S.C. § 112, second paragraph. Claims 34, 35, 38, 39, 45, and 50 are canceled, rendering their rejection moot. Applicants respectfully traverse the rejection of claims 40-44 and 46-49.

The Office Action mailed November 13, 2000 faults the claims for not defining the recited *P. haemolytica* bacterium as attenuated. Each of claims 40-44 and 46-49, as well as each of new claims 51-62, now specifies a mutation "which attenuates the bacterium."

The Markush group recited in now-canceled claim 34 is said to be improper. Applicants believe the Markush group is proper. To advance prosecution, however, members of the Markush group have been placed into separate independent claims (46-49).

Applicants respectfully request withdrawal of the rejection.

The Rejection of Claims 34, 35, and 38-47 Under 35 U.S.C. § 112, first paragraph

Claims 34, 35, and 38-47 stand rejected under 35 U.S.C. § 112, first paragraph, as not enabled for their full scope. The Office Action asserts that the specification enables only the production of aroA mutants and does not enable strains of *Pasteurella* haemolytica with any mutations in the *aro*A, *Pha*I, leukotoxin C, and leukotoxin A genes and use of these strains as

vaccines. Claims 34, 35, 38, 39, and 45 are canceled, and their rejection is now moot. Applicants respectfully traverse the rejection of claims 40-44, 46, and 47.

The rejected claims recite a vaccine comprising a *P. haemolytica* bacterium comprising a mutation in an *aro*A (claims 40-44), leukotoxin C (claim 46), or leukotoxin A (claim 47) gene. The Office Action faults the claims for not requiring an effect of the recited mutation. Each of claims 40-44, 46, and 47 has been amended to require that the recited mutation "attenuates the bacterium."

The Office Action also faults the specification for not providing evidence that the claimed vaccines are capable of inducing protective immunity *P. haemolytica* infection. A Declaration of inventors Robert E. Briggs and Fred M. Tatum under 37 C.F.R. § 1.132 accompanies this amendment. The Declaration describes two experiments in which a vaccine comprising a *P. haemolytica* bacterium which comprises a leukotoxin A mutation (a deletion of amino acids 34 to 378) was used to vaccinate calves against *P. haemolytica* infection. This vaccine meets the requirements of claim 47, which recites a vaccine comprising a *P. haemolytica* bacterium comprising a mutation in a leukotoxin A gene. The experiments demonstrate that the vaccine provides a protective effect against *P. haemolytica* challenge in the vaccinated calves, both under laboratory and field conditions, even without inclusion of an adjuvant.

The experiments described in the Declaration demonstrate that a vaccine comprising a *P. haemolytica* bacterium comprising a mutation in the leukotoxin A gene works as Applicants taught it would in the specification. These experiments also support the efficacy of vaccines comprising a *P. haemolytica* bacterium comprising a mutation in a leukotoxin B, C, or D gene. Leukotoxin C gene function is required for lytic activity of leukotoxin A, and leukotoxin B and D gene function are required for leukotoxin A secretion. Cruz *et al.*, page 1933, column 1, end

of first paragraph. Because proper functioning of leukotoxin A is dependent on the proper functioning of the leukotoxin B, C, and D genes, a vaccine comprising a *P. haemolytica* bacterium comprising a mutation in any of the leukotoxin B, C, or D genes would be expected to provide protection against *P. haemolytica* challenge.

This argument applies with equal force to newly added claims 51-62. Applicants respectfully request withdrawal of the rejection.

The Rejection of Claims 34, 35, 38-40, and 48-50 Under 35 U.S.C. § 112, first paragraph

Claims 34, 35, 38-40, and 48-50 stand rejected under 35 U.S.C. § 112, first paragraph. The Office Action asserts that the specification does not provide a written description of the *P. haemolytica* leukotoxin B, leukotoxin D, or neuraminidase genes such that bacteria comprising mutations in these genes could be obtained. Because claims 34, 35, 38, 39, and 50 have been canceled, their rejection is now moot. Claim 40 has been amended to be dependent on claim 41, which is not subject to this rejection. Applicants respectfully traverse the rejection of claims 48 and 49.

Whether the specification meets the written description requirement with respect to claims 48 and 49 is a question of fact. *In re Wertheim*, 541 F.2d 257, 262, 191 U.S.P.Q. 90, 96 (C.C.P.A. 1976). The Patent Office has the burden of presenting evidence or reasons why persons skilled in the art would not recognize that Applicants' specification has provided a description of claims 48 and 49. *In re Wertheim*, 541 F.2d at 265, 191 U.S.P.Q. at 98. The Patent Office has not met this burden.

Amended claims 48 and 49 recite a vaccine comprising a *P. haemolytica* bacterium which comprises a mutation in a leukotoxin B (claim 48) or leukotoxin D (claim 49) gene,

wherein the mutation attenuates the bacterium. The Office Action asserts that disclosure of the nucleotide sequences of the leukotoxin B and leukotoxin D genes is required to establish that Applicants were in possession of the subject matter of claims 48 and 49 when this application was filed. The Office Action cites *Fiers v. Revel*, *Amgen v. Chugai*, and *Fiddes v. Baird* in support of the rejection. The Office Action also faults the specification for not describing "specific means or methods of obtaining a leukotoxin B or D mutant strain of Pasteurella haemolytica that would also serve to induce a protective immune response." Page 14.

Claims 48 and 49 are not directed to novel genes, but to vaccines comprising a *P. haemolytica* bacterium with certain characteristics, *i.e.*, a mutation in a leukotoxin B or leukotoxin D gene which attenuates the bacterium. These characteristics can be recognized without knowledge of the nucleotide sequences of the corresponding genes. The cited cases, which address written description of claimed novel genes, are not relevant.

At the time the present application was filed, the art knew that *P. haemolytica* contained leukotoxin B and leukotoxin D genes. See Chang *et al.*, "Secretion of the *Pasteurella* leukotoxin by *Escherichia coli*," *FEMS Microbiol. Lett. 60*, 169-74, 1989 (Attachment 1), which teaches that leukotoxin B and D are responsible for secretion of leukotoxin A. The art also know how to assay leukotoxin activity, and therefore how to detect mutant bacteria which had lost or altered leukotoxin activity and which, therefore, would be attenuated (*i.e.*, non-virulent). See, for example, page 1938 of Cruz *et al.* Leukotoxin B or D mutations (including mutations in the regulatory regions of the leukotoxin operon) also could be detected, for example, by observing altered secretion of leukotoxin A or altered size of levels of leukotoxin proteins. Chang *et al.*, 1989. There is simply no legal requirement that the specification describe the nucleotide sequences of either the coding or regulatory regions of these genes to provide a written

description of the subject matter of claims 48 and 49, *i.e.*, bacteria with mutations in known genes.

Even if, *arguendo*, nucleotide sequences were properly required to show that Applicants had possession of the subject matter of claims 48 and 49, Applicants need not have provided these sequences in the specification. The nucleotide sequence of the entire *P. haemolytica* leukotoxin operon, including the *lkt*B and *lkt*D genes, had long been known in the art when this application was filed. See Figure 3 of Highlander *et al.*, 1989, "DNA Sequence of the *Pasteurella haemolytica* Leukotoxin Gene Cluster," *DNA 8*, 15-28,1989 (Attachment 2). Figure 3 provides both the nucleotide sequence of the operon and the amino acid sequences of the encoded proteins and indicates the location of the leukotoxin B and D genes.

The law requires that the specification be considered as a whole when determining whether it describes a particular invention. *In re Wright*, 9 U.S.P.Q.2d 1649, 1651 (Fed. Cir. 1989). Considering the specification as a whole, it is clear that the subject matter of claims 48 and 49 is adequately described. The remarks presented above apply with equal force to newly added dependent claims 57-62.

Applicants respectfully request withdrawal of the rejection.

Respectfully submitted,

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